

Part C. DNase Digestion and Clean-Up for Gene Expression Profiling [Warning: Not for MicroRNA]

Adjust Dnase digestion volume to 50 ul of you have less then 15 ug of RNA.

- 1 Dilute 30 ug of RNA in a volume of 84 ul of Qiagen RNase-free water.
- 2 Add 10ul of 10X Dnase Digestion Buffer (Epicentre D2K-00315).
- 3 Add 2ul of Muring RNase Inhibitor (NEB M0314) -mix by gentle vortexing.
- 4 Add 4ul of Rnase-free Dnase I (Epicentre D2K-00315).
- 5 Mix by inversion or flicking and spin briefly (do not vortex).
- 6 Incubate at 37C for 30 min.
- 7 Add 4.0ul of 0.5 M EDTA pH 8 , vortex and spin down.
- 8 Adjust volume of Dnased-RNA to 100 ul with Qiagen water if necessary.
- 9 Combine DNased digest RNA with 350 ul of buffer RLT and mix by gentle vortexing (don't centrifuge).
- 10 Combine RNA/RLT mixture with 250 ul of 100% ethanol.
- 11 Mix by gentle vortexing (don't centrifuge).
- 12 Apply the sample to an Rneasy MinElute spin column in a 2ml collection tube.
- 13 Close the tube gently and centrifuge for 1 min at >10,000 g.
- 14 Transfer the spin column into a new 2ml collection tube.
- 15 Pipet 500 ul buffer RPE onto the spin column.
(Apply around rim of column to make sure the area is washed)
- 16 Close the tube gently, and centrifuge for 1 min at >10,000 g to wash the column.
- 17 Discard the flow-through and reuse collection tube.
- 18 Pipette 500 ul of 80% ethanol to the Rneasy minelute spin column.
- 19 Close the tube gently, and centrifuge for 1 min at >10,000 g.
- 20 Discard flow-through and collection tube.
- 21 Transfer the Rneasy minelute spin column into a new 2 ml collection tube.
- 22 Close the cap of the spin column, and centrifuge in a microcentrifuge at 16,000 g or max speed for 3 min.
- 23 Discard flow-through and collection tube.
- 24 Repeat steps 21-23 one more times (important to remove any trace of ethanol).
- 25 To elute, transfer the spin column to a new 2 ml capless collection tube.
- 26 Pipette 14 ul Rnase-free water directly onto the center of the silica-gel membrane.
- 27 Close the tube gently, incubate 1 minute on bench and centrifuge for 1 min at max speed to elute
- 28 Measure OD of 1:50 dilution of RNA at 260 nm and 280 nm.
- 29 Freeze at -70C or ship to ORB on dry ice.